

PCT

WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification ⁶ : C07H 19/04, 21/00, A61K 31/70, C12Q 1/68	A3	(11) International Publication Number: WO 99/14226 (43) International Publication Date: 25 March 1999 (25.03.99)
--	----	--

(21) International Application Number: PCT/DK98/00393

(22) International Filing Date: 14 September 1998 (14.09.98)

(30) Priority Data:

1054/97	12 September 1997 (12.09.97)	DK
1492/97	19 December 1997 (19.12.97)	DK
0061/98	16 January 1998 (16.01.98)	DK
0286/98	3 March 1998 (03.03.98)	DK
0585/98	29 April 1998 (29.04.98)	DK
60/088,309	5 June 1998 (05.06.98)	US
PA 1998 00750	8 June 1998 (08.06.98)	DK
PA 1998 00982	28 July 1998 (28.07.98)	DK

(71) Applicant (for all designated States except US): EXIQON A/S
[DK/DK]; Bygstubben 9, DK-2950 Vedbæk (DK).

(72) Inventors; and

(75) Inventors/Applicants (for US only): WENGEL, Jesper
[DK/DK]; Rugmarken 48, DK-5260 Odense S. (DK).
NIELSEN, Poul [DK/DK]; Elmevangen 6, DK-7200
Grindsted (DK).(74) Agent: PLOUGMANN, VINGTOFT & PARTNERS; Sankt
Annæ Plads 11, P.O. Box 3007, DK-1021 Copenhagen
(DK).(81) Designated States: AL, AM, AT, AT (Utility model), AU, AZ,
BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, CZ (Utility
model), DE, DE (Utility model), DK, DK (Utility model),
EE, EE (Utility model), ES, FI, GB, GE, GH, GM, HR,
HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR,
LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ,
PL, PT, RO, RU, SD, SE, SG, SI, SK, SK (Utility model),
SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW,
ARIPO patent (GH, GM, KE, LS, MW, SD, SZ, UG, ZW),
Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM),
European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR,
GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF,
BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN,
TD, TG).

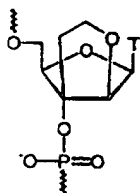
Published

With international search report.

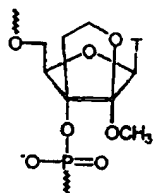
(88) Date of publication of the international search report:

5 August 1999 (05.08.99)

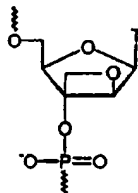
(54) Title: BI- AND TRI-CYCLIC NUCLEOSIDE, NUCLEOTIDE AND OLIGONUCLEOTIDE ANALOGUES



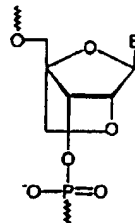
V



X



Y



ZT: B = thymine-1-yl
ZU: B = uracil-1-yl
ZG: B = guanine-9-yl
ZC: B = cytosine-1-yl
ZA: B = adenine-9-yl
ZMoC: B = 5-methylcytosine-1-yl

(57) Abstract

The present invention relates to novel bicyclic and tricyclic nucleoside and nucleotide analogues of formula (I) as well as to oligonucleotides comprising such elements. The nucleotide analogues, LNAs (Locked Nucleoside Analogues), are able to provide valuable improvements to oligonucleotides with respect to affinity and specificity towards complementary RNA and DNA oligomers. The novel type of LNA modified oligonucleotides, as well as the LNAs as such, are useful in a wide range of diagnostic applications as well as therapeutic applications. Among these can be mentioned antisense applications, PCR applications, strand displacement oligomers, as substrates for nucleic acid polymerases, as nucleotide based drugs, etc. The present invention also relates to such applications.

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AL	Albania	ES	Spain	LS	Lesotho	SI	Slovenia
AM	Armenia	FI	Finland	LT	Lithuania	SK	Slovakia
AT	Austria	FR	France	LU	Luxembourg	SN	Senegal
AU	Australia	GA	Gabon	LV	Latvia	SZ	Swaziland
AZ	Azerbaijan	GB	United Kingdom	MC	Monaco	TD	Chad
BA	Bosnia and Herzegovina	GE	Georgia	MD	Republic of Moldova	TG	Togo
BB	Barbados	GH	Ghana	MG	Madagascar	TJ	Tajikistan
BE	Belgium	GN	Guinea	MK	The former Yugoslav Republic of Macedonia	TM	Turkmenistan
BF	Burkina Faso	GR	Greece	ML	Mali	TR	Turkey
BG	Bulgaria	HU	Hungary	MN	Mongolia	TT	Trinidad and Tobago
BJ	Benin	IE	Ireland	MR	Mauritania	UA	Ukraine
BR	Brazil	IL	Israel	MW	Malawi	UG	Uganda
BY	Belarus	IS	Iceland	MX	Mexico	US	United States of America
CA	Canada	IT	Italy	NE	Niger	UZ	Uzbekistan
CF	Central African Republic	JP	Japan	NL	Netherlands	VN	Viet Nam
CG	Congo	KE	Kenya	NO	Norway	YU	Yugoslavia
CH	Switzerland	KG	Kyrgyzstan	NZ	New Zealand	ZW	Zimbabwe
CI	Côte d'Ivoire	KP	Democratic People's Republic of Korea	PL	Poland		
CM	Cameroon	KR	Republic of Korea	PT	Portugal		
CN	China	KZ	Kazakhstan	RO	Romania		
CU	Cuba	LC	Saint Lucia	RU	Russian Federation		
CZ	Czech Republic	LI	Liechtenstein	SD	Sudan		
DE	Germany	LK	Sri Lanka	SE	Sweden		
DK	Denmark	LR	Liberia	SG	Singapore		
EE	Estonia						

INTERNATIONAL SEARCH REPORT

International Application No.

PCT/DK 98/00393

A. CLASSIFICATION OF SUBJECT MATTER

IPC 6 C07H19/04 C07H21/00 A61K31/70 C12Q1/68

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 6 C07H A61K C12Q

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	<p>P.NIELSEN ET AL.: "A Novel Class of Conformationally Restricted Oligonucleotide Analogues : Synthesis of 2',3'-Bridged Monomers and RNA-Selective Hybridisation."</p> <p>JOURNAL OF THE CHEMICAL SOCIETY, CHEMICAL COMMUNICATIONS.,</p> <p>no. 9, 7 May 1997, LETCHWORTH GB,</p> <p>pages 825-826, XP002046993</p> <p>cited in the application</p> <p>see page 825, compounds 5 and x</p> <p>---</p> <p>-/--</p>	<p>1-75,</p> <p>80-140</p>

☒ Further documents are listed in the continuation of box C.

☐ Patent family members are listed in annex.

* Special categories of cited documents :

- "A" document defining the general state of the art which is not considered to be of particular relevance
- "E" earlier document but published on or after the international filing date
- "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- "O" document referring to an oral disclosure, use, exhibition or other means
- "P" document published prior to the international filing date but later than the priority date claimed

- "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
- "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
- "&" document member of the same patent family

Date of the actual completion of the international search

8 March 1999

Date of mailing of the international search report

06.05.1999

Name and mailing address of the ISA

European Patent Office, P.B. 5818 Patentlaan 2
NL - 2280 HV Rijswijk
Tel. (+31-70) 340-2040, Tx. 31 651 epo nl,
Fax: (+31-70) 340-3016

Authorized officer

SCOTT, J

INTERNATIONAL SEARCH REPORT

International Application No

PCT/DK 98/00393

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	V.E.MARQUEZ ET AL.: "Nucleosides with a Twist. Can Fixed Forms of Sugar Ring Pucker Influence Biological Activity in Nucleosides and Oligonucleotides ?" JOURNAL OF MEDICINAL CHEMISTRY., vol. 39, no. 19, 13 September 1996, WASHINGTON US, pages 3739-3747, XP002094300 see the whole document ---	1-75, 80-140
Y	M.BOLLI ET AL.: "Bicyclo-DNA : A Hoogsteen-Selective Pairing System." CHEMISTRY AND BIOLOGY, no. 3, March 1996, pages 197-206, XP002094301 see the whole document ---	1-75, 80-140
A	C.G.YANNOPOULOS ET AL.: "2',3'-Cyclopropanated Nucleoside Dimers." SYNLETT., no. 4, 1997, STUTTGART DE, pages 378-380, XP002046994 cited in the application see the whole document ---	1
A	CHEMICAL ABSTRACTS, vol. 70, no. 1, 6 January 1969 Columbus, Ohio, US; abstract no. 3737b, G.ZIGEUNER ET AL.: "Heterocycles. XVI. 1,4-Dimethyl-3-acetoxy-7-acetamido-2-oxabi cyclo(2.2.1)heptane." page 343; column 1; XP002046995 see abstract & MONATSCH. CHEM., vol. 99, no. 5, 1968, pages 2111-2120, ---	1
P,X	R.KUMAR ET AL.: "The first Analogues of LNA (Locked Nucleic Acids) : Phosphorothioate-LNA and 2'-Thio-LNA." BIOORGANIC AND MEDICINAL CHEMISTRY LETTERS, no. 8, 1998, pages 2219-2222, XP002094302 see the whole document ---	1-75, 80-140

-/--

INTERNATIONAL SEARCH REPORT

Int'l Application No

PCT/DK 98/00393

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,X	S.K.SINGH ET AL.: "LNA (Locked Nucleic Acids) : Synthesis and High-Affinity Nucleic Acid Recognition." CHEMICAL COMMUNICATIONS., no. 4, 21 February 1998, CIETY OF CHEMISTRY GB, pages 455-456, XP002094303 see the whole document ---	1-75, 80-140
P,X	A.A.KOSHKIN ET AL.: "LNA (Locked Nucleic Acids) : Synthesis of the Adenine, Cytosine, Guanine, 5-Methylcytosine, Thymine and Uracil Bicyclonucleoside Monomers, Oligomerisation, and the Unprecedented Nucleic Acid Recognition." TETRAHEDRON, vol. 54, 1998, pages 3607-3630, XP002094304 see the whole document ---	1-75, 80-140
T	P.HERDEWIJN: "Targeting RNA with Conformationally Restricted Oligonucleotides." LIEBIGS ANNALEN: ORGANIC AND BIOORGANIC CHEMISTRY., no. 9, September 1996, ISHERS US, pages 1337-1348, XP002094305 see the whole document -----	1

INTERNATIONAL SEARCH REPORT

International application No.
PCT/DK 98/00393

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
2. ☐ Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:
1-75, 80-140 partially

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

1. Claims: 1-75,80-140 partially

A nucleoside with one pair of geminal substituents forming a biradical between the 2' and 4'positions (LNA); oligomers containing these LNAs; uses of both the LNA and the oligomer containing the LNA; conjugates of the oligomer; solid surfaces with LNA or oligomers containing them.

2. Claims: 1-28,43-68,76-82,93-140 partially

A nucleoside with one pair of geminal substituents forming a biradical between the 2' and 3'positions (LNA); oligomers containing these LNAs; uses of both the LNA and the oligomer containing the LNA; conjugates of the oligomer; solid surfaces with LNA or oligomers containing them.

3. Claims: 1-28,43-68,93-140 partially

A nucleoside with one pair of geminal substituents forming a biradical between the 3' and 4'positions (LNA); oligomers containing these LNAs; uses of both the LNA and the oligomer containing the LNA; conjugates of the oligomer; solid surfaces with LNA or oligomers containing them.

4. Claims: 1-28,43-68,93-140 partially

A nucleoside with one pair of geminal substituents forming a biradical between the 3' and 5'positions (LNA); oligomers containing these LNAs; uses of both the LNA and the oligomer containing the LNA; conjugates of the oligomer; solid surfaces with LNA or oligomers containing them.

5. Claims: 1-28,43-68,93-140 partially

A nucleoside with one pair of geminal substituents forming a biradical between the 1' and 4'positions (LNA); oligomers containing these LNAs; uses of both the LNA and the oligomer containing the LNA; conjugates of the oligomer; solid surfaces with LNA or oligomers containing them.

6. Claims: 1-28,43-68,93-140 partially

A nucleoside with one pair of geminal substituents forming a biradical between the 1' and 2'positions (LNA); oligomers containing these LNAs; uses of both the LNA and the oligomer containing the LNA; conjugates of the oligomer; solid surfaces with LNA or oligomers containing them.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

7. Claims: 1-140 partially

A nucleoside with two pairs of geminal substituents forming a biradical (LNA); oligomers containing these LNAs; uses of both the LNA and the oligomer containing the LNA; conjugates of the oligomer; solid surfaces with LNA or oligomers containing them.

8. Claims: 1-11,39-59,97,103,105-140 partially

Oligomers containing LNAs with one pair of geminal substituents forming a biradical between the 1' and 5' positions; uses of both the LNA and the oligomer containing the LNA; conjugates of the oligomer; solid surfaces with LNA or oligomers containing them.

9. Claims: 1-11,39-59,97,103,105-140 partially

Oligomers containing LNAs with one pair of geminal substituents forming a biradical between the 1' and 3' positions; uses of both the LNA and the oligomer containing the LNA; conjugates of the oligomer; solid surfaces with LNA or oligomers containing them.

10. Claims: 1-11,39-59,97,103,105-140 partially

Oligomers containing LNAs with one pair of geminal substituents forming a biradical between the 2' and 5' positions; uses of both the LNA and the oligomer containing the LNA; conjugates of the oligomer; solid surfaces with LNA or oligomers containing them.

11. Claims: 1-11,39-59,97,103,105-140 partially

Oligomers containing these LNAs with one pair of geminal substituents forming a biradical between the 4' and 5' positions; uses of both the LNA and the oligomer containing the LNA; conjugates of the oligomer; solid surfaces with LNA or oligomers containing them.